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Relationships among serum receptor of nuclear factor-κB ligand, osteoprotegerin, high-sensitivity C-reactive protein, and bone mineral density in postmenopausal women: osteoimmunity versus osteoinflammatory

Iraj Nabipour, MD,[1] Bagher Larijani, MD,[2] Katayoun Vahdat, MD,[3] Majid Assadi, MD,[4] Seyed Mojtaba Jafari, MSc,[1] Esmaeil Ahmadi, MD,[1] Ali Movahed, PhD,[1] Farzad Moradhaseli, MD,[1] Zahra Sanjdideh, MSc,[1] Narges Obeidi, MSc,[1] and Zahra Amiri, MSc[1]

Abstract

Objective: The aim of this study was to investigate the correlations among circulating osteoprotegerin (OPG), the receptor activator of nuclear factor-κB ligand (RANKL), high-sensitivity C-reactive protein (hsCRP), and bone mineral density (BMD) in healthy postmenopausal women.

Methods: In a population-based study, highly specific enzyme-linked immunosorbent assay methods were used to evaluate the sera of 382 healthy Iranian postmenopausal women (mean age ± SD, 58.7 ± 7.5 y) for RANKL, OPG, hsCRP, degradation products of C-terminal telopeptides of type I collagen, and osteocalcin. BMD was determined for the lumbar spine (L2-L4) and the proximal femur using dual-energy x-ray absorptiometry.

Results: Circulating levels of OPG ($r = 0.30$, $P < 0.001$) and the RANKL/OPG ratio ($r = -0.17$, $P < 0.001$) were significantly associated with age. The geometric mean of hsCRP was 1.89 mg/L (SE, 1.05) in the population studied. There was a significant correlation between log(hsCRP) levels and body mass index (BMI; $r = 0.36$, $P < 0.001$). Multivariate linear analyses revealed that age ($\beta = -0.295$, $P < 0.001$), BMI ($\beta = 0.464$, $P < 0.001$), RANKL ($\beta = -0.105$, $P = 0.014$), and OPG ($\beta = 0.098$, $P = 0.029$) were the independent determinants for lumbar BMD ($R^2 = 0.35$). Age ($\beta = -0.250$, $P < 0.001$), BMI ($\beta = 0.486$, $P < 0.001$), and RANKL ($\beta = -0.110$, $P = 0.009$) were independently correlated with femoral neck BMD ($R^2 = 0.36$). Age- and BMI-adjusted analysis by quartiles of log-transformed hsCRP did not reveal an association with BMD, serum levels of biochemical markers of bone turnover, RANKL, or OPG.

Conclusions: The circulating levels of the RANKL/OPG osteoimmunity system have an association with BMD, but subclinical systemic inflammation may not be involved in bone mass in healthy postmenopausal women.

Key Words: Receptor activator of nuclear factor-κB ligand – Osteoprotegerin – C-reactive protein – Inflammation – Bone mineral density.

A balance of the two peptides produced by osteoblasts, osteoprotegerin (OPG) and the receptor activator of nuclear factor-κB ligand (RANKL), is essential for osteoclast function and bone remodeling.[1] RANKL is a cytokine belonging to the tumor necrosis factor (TNF) family that binds to membrane-bound receptor RANK on osteoclasts and promotes differentiation of marrow cells through various stages to multinucleated osteoclasts that resorb bone.[2] OPG acts as a blocking receptor by binding to RANKL and preventing it from binding to RANK.[3,4]

Most of the factors that induce RANKL expression by osteoblasts also regulate OPG expression.[5] Systemic factors such as glucocorticoids, immunosuppressants, and estrogen deficiency and continuous exposure of parathyroid hormone increase the RANKL/OPG ratio.[1] Thus, the relative concentration of RANKL and OPG in bone is a major determinant of bone health.

The RANK/RANKL/OPG system has also been shown to modulate dendritic cells and activate T cells, as well as to promote B-cell maturation and antibody response, which suggests that this system plays an important role in both the

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From the [1]Department of Endocrine and Metabolic Diseases, The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr; [2]Tehran Endocrine Research Center, Tehran University of Medical Sciences, Tehran; and Departments of [3]Infectious Diseases and [4]Nuclear Medicine, The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Islamic Republic of Iran.

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Address correspondence to: Iraj Nabipour, MD, The Persian Gulf Tropical Medicine Research Center, Boostan 19 Alley, Imam Khomeini Street, Bushehr, Islamic Republic of Iran. E-mail: inabipour@gmail.com

skeletal and immune systems.[6] This osteoimmunological system configures interesting molecular links between bone remodeling, immunity, and inflammation.[7,8]

Measurement of the concentration of C-reactive protein (CRP), an acute-phase reactant, has been used as a sensitive marker of chronic, low-grade systemic inflammation. CRP is produced by the liver under induction by interleukin (IL) 1, IL-6, and TNF-α.[7] The mechanism linking high-sensitivity CRP (hsCRP) and bone metabolism has not been completely clarified, but substantial evidence that certain inflammatory cytokines, including IL-1, IL-6, and TNF-α, are likely to be involved is suggested.[9] Previous studies also reported that the serum hsCRP level was a significant predictor of osteoporotic fracture.[10,11] Therefore, chronic, low-grade inflammation could be considered an important factor in lower bone mineral density (BMD) in postmenopausal osteoporosis.

Investigations in humans under various conditions and disease states have examined serum levels of OPG and/or RANKL with conflicting results.[12] However, the relationship of the RANKL/OPG system, subclinical inflammation, and BMD in healthy postmenopausal women has not been adequately examined. This is of primary importance because the interaction of osteoimmunity and chronic, low-grade inflammation on bone remodeling remains to be clarified. Thus, the aim of this study was to investigate the relationships between serum concentrations of OPG, RANKL, OPG/RANKL ratio, hsCRP, and BMDs in Iranian postmenopausal women.

METHODS

Community sampling and physical examinations

The study design has been described previously.[13] However, in brief, participants in the present study were an age-stratified random sample of postmenopausal women who participated in the extension part of the Iranian Multicentral Osteoporosis Study. They were randomly selected from 13 clusters in Bushehr port (the center of Bushehr Province, which has the longest border with the Persian Gulf). All were community dwelling and ambulatory.

The following exclusion criteria were used: (*1*) the known presence of generalized bone diseases including hyperparathyroidism, hypoparathyroidism, thyroid disorders, rheumatoid arthritis, Cushing disease, and steroid-induced osteoporosis; renal osteodystrophy; or other metabolic diseases; (*2*) a history of malignant diseases and liver diseases; (*3*) drug addiction; and (*4*) restriction to bed rest within the last 2 weeks after an illness or complete bed rest for 3 months.

Height and weight were measured using a stadiometer. Heavy outer garments and shoes were removed before height and weight were measured. Waist circumference was defined at the midway level between the costal margins and the iliac crests. Hip circumference was measured at the level of the greater trochanters.

BMD was determined for the lumbar spine (L2-L4) and proximal femur (neck) using dual-energy x-ray absorptiometry on an Osteocore II bone densitometer (Osteocore II Osteodensitometer; Medilink, France). To eliminate operator discrepancies, the same operator tested all the women during the study. Duplicate measurements were obtained from 30 women who were agreeable to undergoing a repeat assessment on the same day, and the precision errors were calculated using the root mean square method. The coefficients of variation (CVs; precision) of measurements of the lumbar spine and femoral neck were 0.8% and 1.6%, respectively.

Laboratory measurements

Measurement of CRP by an hsCRP assay, CRP HS enzyme-linked immunosorbent assay (ELISA; DRG International, Inc., Mountainside, NJ), was conducted. The detectable concentration of the CRP HS ELISA assay was estimated to be 0.1 mg/L. In addition, the functional sensitivity was determined to be 0.1 mg/L (as determined with interassay %CV <20%).

Serum OPG levels were measured using an ELISA commercial kit (Biomedica Gruppe, Vienna, Austria). The detection limit of the assay was 2.8 pg/mL. The mean intra-assay and interassay CVs of the OPG assay were 4% to 10% and 7% to 8%, respectively.

RANKL levels were measured using an ELISA with an additional enhancement system (ampli-sRANKL; Biomedica Gruppe). The detection limit of the assay was 0.4 pg/mL. The mean intra-assay and interassay CVs of the RANKL assay were 8% to 9% and 6% to 3%, respectively.

The Serum CrossLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) was used to quantify the degradation products of C-terminal telopeptides of type I collagen in sera. The intra-assay CVs for the low (0.242 ng/mL), medium (0.375 ng/mL), and high (0.476 ng/mL) values were 5.4%, 5.0%, and 5.1%, respectively.

The N-MID Osteocalcin ELISA (Nordic Bioscience Diagnostics A/S) was used for the quantitative measurement of osteocalcin in sera. The intra-assay CVs for the low (7.0 ng/mL), medium (21.8 ng/mL), and high (43.2 ng/mL) values were 3.4%, 2.0%, and 2.4%, respectively.

Statistical analysis

The distribution of variables was studied using probability plots and the Shapiro-Wilk test. We found that log transformation of CrossLaps, osteocalcin, hsCRP, OPG, and RANKL gave a better fit to a gaussian distribution. The geometric mean for those biochemical variables was defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10.

Pearson's correlation analysis was used to study the relationships among the anthropometric and biochemical variables and the BMD measurements. The association of log(hsCRP) quartiles (Qs) with BMD, the RANKL/OPG system, and markers of bone turnover was also determined using analysis of covariance.

Multiple linear models were used to explore the effects of the RANKL/OPG system or hsCRP concentrations on BMD after adjustment for covariates. Age and body mass index (BMI) were considered as covariates in the linear regression

TABLE 1. *Basic characteristics of bone-related variables in an Iranian postmenopausal population (382 participants)*

	Mean	SD
Age, y	58.71	7.50
Body mass index, kg/m^2	28.34	4.73
Waist-to-hip ratio	0.92	0.06
RANKL, pg/mL[a]	1.59	3.00
OPG, pg/mL[a]	72.44	1.55
RANKL/OPG ratio	0.04	0.05
CrossLaps, ng/mL[a]	591.56	1.69
Osteocalcin, ng/mL[a]	11.45	1.85
hsCRP, mg/L[a]	1.89	2.84
Femoral neck BMD, g/cm^2	0.844	0.184
Wards triangle BMD, g/cm^2	0.608	0.252
Trochanter BMD, g/cm^2	0.632	0.148
Lumbar BMD, g/cm^2	0.946	0.186

Data are means and SDs. RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; hsCRP, high-sensitivity C-reactive protein; BMD, bone mineral density.
[a]Geometric mean (SD).

models because these variables significantly correlated with BMDs in univariate analyses. Multivariate linear analyses were also performed to investigate the independent effect of age, BMI, RANKL, OPG, and hsCRP on BMD at the lumbar or femoral neck areas in separate models. All the independent variables were entered at once. A P value less than 0.05 was accepted as the value of significance.

Statistical analysis was performed with an IBM computer using the SPSS 9.05 statistical software package (SPSS Inc., Chicago, IL).

RESULTS

The characteristics of the study participants are shown in Table 1. The prevalence of consumption of oral calcium, vitamin D supplementation, and use of hormone therapy was 5.7%, 4.2%, and 1.0%, respectively. According to the World Health Organization criteria, 150 (39.3%) and 127 (33.2%) women were considered osteopenic/osteoporotic at the lumbar and femoral neck sites, respectively.[14]

BMD at the lumbar spine and femoral neck decreased progressively as age increased ($P < 0.001$). There were significant positive associations between BMI and BMD at all sites ($P < 0.001$). However, there was no significant correlation between waist-to-hip ratio and BMD at the lumbar and femoral sites ($P > 0.05$).

The geometric mean of CRP was 1.89 mg/L (SE, 1.05) in the studied population. Qs for the population distribution for CRP were as follows: Q1, 0.03-1.05 mg/L; Q2, 1.06-2.00 mg/L; Q3, 2.01-4.22 mg/L; and Q4, 4.23-26.91 mg/L. Serum levels of log(hsCRP) had a significant correlation with BMI ($r = 0.36$, $P < 0.001$) and waist-to-hip ratio ($r = 0.16$, $P = 0.002$).

The OPG ($r = 0.30$, $P < 0.001$) and RANKL/OPG ratio ($r = -0.17$, $P < 0.001$) levels were significantly associated with age. Age-adjusted serum levels of OPG showed no association with BMD at the lumbar and femoral sites, but age-adjusted RANKL and the RANKL/OPG ratio were negatively associated with BMD at the lumbar ($r = -0.12$, $P = 0.019$ and $r = -0.13$, $P = 0.007$, respectively) and femoral neck ($r = -0.13$, $P = 0.012$ and $r = -0.12$, $P = 0.020$, respectively) sites.

In multiple regression models, serum concentrations of RANKL (negatively) and the RANKL/OPG ratio (positively) were significantly related to BMD at the lumbar and femoral neck sites, and OPG was found to be significantly correlated with lumbar BMD (Table 2). The correlations of the RANKL/OPG regulatory system with BMD remained when log(hsCRP) was considered as a covariate to the baseline regression models (data not shown).

Multivariate linear analyses were also performed to investigate the independent effect of age, BMI, RANKL, OPG, and hsCRP on BMD at the lumbar or femoral neck areas.

The results revealed that age ($\beta = -0.295$, $P < 0.001$), BMI ($\beta = 0.464$, $P < 0.001$), RANKL ($\beta = -0.105$, $P = 0.014$), and OPG ($\beta = 0.098$, $P = 0.029$) were the independent determinants for lumbar BMD ($R^2 = 0.35$). Age ($\beta = -0.250$, $P < 0.001$), BMI ($\beta = 0.486$, $P < 0.001$), and RANKL ($\beta = -0.110$, $P = 0.009$) were independently correlated with femoral neck BMD ($R^2 = 0.36$).

There was no significant age- and BMI-adjusted linear correlation between log(hsCRP) concentrations and BMD in either the lumbar spine or the proximal femur (Table 2).

Age- and BMI-adjusted analysis by Qs of log(hsCRP) did not reveal an association with BMD, RANKL, OPG, RANKL/OPG ratio levels, or circulating bone turnover marker concentrations (data not shown).

In addition, the RANKL, OPG, and RANKL/OPG serum levels were not related to logarithmically transformed serum CrossLaps and osteocalcin concentrations.

DISCUSSION

We found that serum RANKL levels were negatively related to BMD at the femoral neck and lumbar spine, and circulating OPG concentrations were positively correlated to

TABLE 2. *Age- and BMI-adjusted standardized β coefficients for the multiple linear regressions of circulating levels of RANKL/OPG regulatory system and hsCRP with bone mineral densities in an Iranian postmenopausal population (382 women)*

	Lumbar spine		Trochanter		Femoral neck		Wards triangle	
	β	*P*	β	*P*	β	*P*	β	*P*
RANKL	−0.102	0.017	−0.060	0.162	−0.110	0.009	−0.018	0.663
OPG	0.101	0.022	−0.004	0.924	0.027	0.532	0.054	0.204
RANKL/OPG ratio	−0.131	0.003	−0.050	0.210	−0.114	0.008	−0.034	0.421
hsCRP	0.004	0.932	0.031	0.486	0.012	0.780	−0.016	0.710

All the variables were log transformed for analyses. BMI, body mass index; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; hsCRP, high-sensitivity C-reactive protein.

BMD at the lumbar spine, independent of age and BMI, in healthy, Iranian postmenopausal women.

The OPG/RANKL/RANK cytokine system is essential for osteoclast biology, and accumulating evidence suggests the role of this system in many clinically relevant metabolic bone diseases in humans.[1]

In postmenopausal osteoporosis, polymorphisms in the TNF superfamily member 11 gene (that codes for RANKL) promoter were associated with BMD.[15] The OPG and RANKL gene polymorphism was identified as a genetic factor associated with BMD of the lumbar spine in Korean women.[16] Recently, denosumab, as a fully human monoclonal antibody that inhibits bone resorption by neutralizing RANKL, showed significantly larger gains in BMD and greater reduction in bone turnover markers compared with alendronate.[17]

Because of these findings, the circulating RANKL and OPG are likely to be involved in the pathogenesis of postmenopausal osteoporosis. However, recent attempts to measure the serum levels of RANKL and OPG as well as BMD measurements in postmenopausal women yielded mixed results. Serum RANKL levels were reported not to be associated with BMD[18-20] and were negatively related with lumbar BMD.[21] However, a low level of soluble RANKL was an independent predictor of nontraumatic fracture.[22] Serum OPG levels have also been found to be positively, negatively, or not related to BMD in postmenopausal women (reviewed in References 12 and 23). A higher OPG concentration was associated with greater femoral neck bone density in postmenopausal women in the Framingham Offspring Study.[24] In the Rancho Bernardo Study,[25] serum OPG levels were significantly positively associated with BMD at the femoral neck, total hip, and lumbar spine in women using estrogen but not in nonusers of estrogen.

Despite clear changes in RANKL expression in marrow cells, Eghbali-Fatourechi et al[26] did not detect differences in circulating RANKL levels in their study, highlighting the importance of assessing the levels of these factors directly in the bone microenvironment. The levels of RANKL in the bone microenvironment may be more relevant, but only a few studies have attempted to investigate this.[12]

These conflicting findings raise the concern that measurement of circulating levels of these cytokines may not be clinically relevant in postmenopausal osteoporosis. Therefore, the relationship between the RANKL/OPG regulatory system and BMD in our study should be interpreted with caution. However, our findings suggest that these regulatory circulating cytokines may be considered potential markers for BMD and postmenopausal osteoporosis.

The RANK/RANKL/OPG regulatory system mediates the effects of systemic factors such as parathyroid hormone, 1,25-dihydroxyvitamin D, and interleukin 1 on bone resorption.[2] Conceptually, it is therefore reasonable to think about the effect of systemic chronic inflammation on bone metabolism in terms of the RANK/RANKL/OPG system. In inflammatory or autoimmune disease states, as part of the autoimmune process, activated T cells produce RANKL and proinflammatory cytokines, all of which can induce RANKL expression in osteoblasts.[7] RANKL gene expression has been found in cells in the synovial tissues of inflamed joints, indicating the importance of RANKL in the pathogenesis of joint damage.[2] Recently, a significant correlation was reported between serum RANKL and CRP in rheumatoid arthritis.[27] However, no publication on the relationship between serum RANKL and BMD in healthy individuals with subclinical inflammation could be found. In the present study, for the first time, we showed that beyond the RANKL/OPG regulatory system, chronic low-grade inflammation did not account for any variance in BMD at all sites.

There is little information about the serum levels of RANKL/OPG and circulating hsCRP in postmenopausal women. The only published study reported that serum OPG values were elevated regardless of high bone turnover, in a close relationship with the serum CRP measurement in long-lived participants (age range, 85-110 y).[28] There was no significant correlation between RANKL or OPG and hsCRP in the present study. Therefore, chronic low-grade inflammation would not seem to be an important factor influencing these cytokine serum concentrations.

TNF has been indirectly implicated in postmenopausal and inflammation-associated bone loss. Interestingly, TNF (like RANKL) can induce the sequential expression of nuclear factor κB, c-Fos, and nuclear factor of activated T-cells c1, thereby directly controlling the differentiation of osteoclast precursors into osteoclasts. IL-1 does not activate c-Fos, but in osteoclast precursors in which c-Fos has been activated, for example, by RANKL or TNF, IL-1 can induce osteoclastogenesis directly. This leads to more osteoclast formation using the same NFATc1-activated mechanism as RANKL (reviewed in References 5 and 29). Thus, TNF by inducing c-Fos expression in osteoclast precursors at the sites of inflammation could facilitate a direct induction of osteoclastogenesis by IL-1.[30] This TNF signaling could describe the pathological mechanism of inflammatory conditions, such as rheumatoid arthritis and inflammatory bone diseases, on bone resorption. However, we think that this mechanism does not seem to play a major role in the chronic, low-grade inflammatory state as manifested by high levels of hsCRP in healthy participants because we did not find an association between hsCRP levels and BMD in multiple regression analysis models in healthy postmenopausal women.

Three cross-sectional studies have analyzed the relationship between CRP and BMDs.[31-33] Two of these previous studies[31,32] showed that CRP was not associated with bone mass in healthy postmenopausal women after multivariate analysis. However, in a cross-sectional study, Koh et al[33] showed that higher serum hsCRP levels were associated with lower BMD in a large population of Korean women. As a limitation of this study, the study population was composed of women who visited a health promotion center and may not have been representative of the general population.[34] However, in the present population-based study, we did not

find a significant association between hsCRP and BMD at the lumbar and femoral sites in postmenopausal women residing in a community. Our results are consistent with the other previous studies.[31,32] Pasco et al,[11] who reported circulating hsCRP as an independent predictor of fracture risk in older women, also showed no significant correlation between hsCRP and BMD in their study. These conflicting results suggest that further studies are needed to examine hsCRP as a potential marker of postmenopausal osteoporosis.

The relationship between bone turnover rate and hsCRP has been investigated by three previous studies.[9,11,34] Kim et al[34] found an association between urinary N-terminal telopeptide of type I collagen and serum hsCRP levels in 150 postmenopausal women. Ding et al,[9] in their longitudinal study, reported that a change in the urinary pyridinoline-to-creatinine ratio was positively associated with hsCRP. However, Pasco et al,[11] in their large population-based study, found no significant correlations between hsCRP and serum C-telopeptide as a marker of bone resorption. We also did not find an association between hsCRP and serum CrossLaps and osteocalcin as bone turnover markers.

When the results of the RANKL/OPG regulatory system in serum are analyzed, several limitations should be considered. First, we measured these cytokines in serum, and it is unclear to what extent this correlates to local cytokine production or action within the bone microenvironment. Second, RANKL and OPG are not bone-specific and are produced by various nonskeletal tissues.[5] Third, we acknowledge that the cross-sectional design of our study could not determine if there is a causal relationship between the RANKL/OPG regulatory system and BMD measurements. In addition, we did not measure inflammatory cytokines, including IL-1, IL-6, and TNF-α, that may better configure low-grade, subclinical inflammation in healthy individuals.

Despite these potential limitations, we found a negative relationship between RANKL, the RANKL/OPG ratio, and BMD at the lumbar and femoral neck and a positive relationship between OPG and lumbar BMD. The cross-sectional design prohibits conclusions regarding a causal relation between the circulating RANKL/OPG system and bone health indices examined in this investigation. Therefore, the clinical significance of circulating levels of these cytokines in osteoporosis needs prospective studies in different populations.

CONCLUSIONS

We found a negative relationship between circulating RANKL, the RANKL/OPG ratio, and BMD at the lumbar and femoral neck and a positive relationship between OPG and BMD at the lumbar spine. These findings suggest that the components of the RANKL/OPG osteoimmunological system may provide clinical and biological clues for treatment and prevention of postmenopausal osteoporosis in the future. In this population-based study, serum hsCRP concentrations were not associated with BMD at either site, circulating levels of RANKL/OPG system, and markers of bone turnover. Therefore, subclinical systemic inflammation may not be involved in the pathogenesis of osteoporosis. In conclusion, it seems that the osteoimmunity system is more important than subclinical inflammation in bone mass of healthy postmenopausal women.

REFERENCES

1. Hofbauer LC, Kuhne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. *J Musculoskelet Neuronal Interact* 2004; 4:268-275.
2. Gallagher JC. Advances in bone biology and new treatments for bone loss. *Maturitas* 2008;60:65-69.
3. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998;12:1260-1268.
4. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; 89:309-319.
5. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008;473:139-146.
6. Ueland T, Yndestad A, Oie E, et al. Dysregulated osteoprotegerin/RANK ligand/RANK axis in clinical and experimental heart failure. *Circulation* 2005;111:2461-2468.
7. Ginaldi L, Di Benedetto MC, De Martinis M. Osteoporosis, inflammation and ageing. *Immun Ageing* 2005;2:14.
8. McCormick RK. Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility. *Altern Med Rev* 2007;12:113-145.
9. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. *J Clin Endocrinol Metab* 2008;93:1952-1958.
10. Schett G, Kiechl S, Weger S, et al. High-sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. *Arch Intern Med* 2006;166:2495-2501.
11. Pasco JA, Kotowicz MA, Henry MJ, et al. High-sensitivity C-reactive protein and fracture risk in elderly women. *JAMA* 2006;296:1353-1355.
12. Kearns AE, Khosla S, Kostenuik PJ. Receptor activator of nuclear factor κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev* 2008;29:155-192.
13. Nabipour I, Larijani B, Beigi S, et al. Relationship among insulinlike growth factor I concentrations, bone mineral density, and biochemical markers of bone turnover in postmenopausal women: a population-based study. *Menopause* 2008;15:934-939.
14. World Health Organization. Assesment of fracture risk and its application to screening for postmenopausal osteoporosis. World Health Organization, Geneva. Technical Report Series. 1994;843:1-129.
15. Mencej S, Albagha OM, Prezelj J, Kocjan T, Marc J. Tumour necrosis factor superfamily member 11 gene promoter polymorphisms modulate promoter activity and influence bone mineral density in postmenopausal women with osteoporosis. *J Mol Endocrinol* 2008;40:273-279.
16. Kim JG, Kim JH, Kim JY, et al. Association between osteoprotegerin (OPG), receptor activator of nuclear factor-κB (RANK), and RANK ligand (RANKL) gene polymorphisms and circulating OPG, soluble RANKL levels, and bone mineral density in Korean postmenopausal women. *Menopause* 2007;14:913-918.
17. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res* 2009;24:153-161.
18. Liu JM, Zhao HY, Ning G, et al. Relationships between the changes of serum levels of OPG and RANKL with age, menopause, bone biochemical markers and bone mineral density in Chinese women aged 20-75. *Calcif Tissue Int* 2005;76:1-6.
19. Mezquita-Raya P, de la Higuera M, Garcia DF, et al. The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women. *Osteoporos Int* 2005;16:1368-1374.
20. Kim JG, Kim JH, Lee DO, et al. Changes in the serum levels of osteoprotegerin and soluble receptor activator for nuclear factor κB ligand after estrogen-progestogen therapy and their relationships with changes in bone mass in postmenopausal women. *Menopause* 2008; 15:357-362.

21. Uemura H, Yasui T, Miyatani Y, et al. Circulating profiles of osteoprotegerin and soluble receptor activator of nuclear factor kappaB ligand in post-menopausal women. *J Endocrinol Invest* 2008; 31:163-168.
22. Schett G, Kiechl S, Redlich K, et al. Soluble RANKL and risk of nontraumatic fracture. *JAMA* 2004;291:1108-1113.
23. Vega D, Maalouf NM, Sakhaee K. Clinical review: The role of receptor activator of nuclear factor-κB (RANK)/RANK ligand/osteoprotegerin: clinical implications. *J Clin Endocrinol Metab* 2007; 92:4514-4521.
24. Samelson EJ, Broe KE, Demissie S, et al. Increased plasma osteoprotegerin concentrations are associated with indices of bone strength of the hip. *J Clin Endocrinol Metab* 2008;93:1789-1795.
25. Stern A, Laughlin GA, Bergstrom J, Barrett-Connor E. The sex-specific association of serum osteoprotegerin and receptor activator of nuclear factor κB legend with bone mineral density in older adults: the Rancho Bernardo study. *Eur J Endocrinol* 2007;156:555-562.
26. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest* 2003;111: 1221-1230.
27. Hein GE, Meister M, Oelzner P, Franke S. sRANKL and OPG in serum and synovial fluid of patients with rheumatoid arthritis in comparison to non-destructive chronic arthritis. *Rheumatol Int* 2008; 28:765-769.
28. Mazziotti G, Amato G, Sorvillo F, et al. Increased serum osteoprotegerin values in long-lived subjects: different effects of inflammation and bone metabolism. *Eur J Endocrinol* 2006;154:373-377.
29. Yamashita T, Yao Z, Li F, et al. NF-κB p50 and p52 regulate receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. *J Biol Chem* 2007;282:18245-18253.
30. Yao Z, Xing L, Qin C, Schwarz EM, Boyce BF. Osteoclast precursor interaction with bone matrix induces osteoclast formation directly by an interleukin-1–mediated autocrine mechanism. *J Biol Chem* 2008;283: 9917-9924.
31. Ganesan K, Teklehaimanot S, Tran TH, Asuncion M, Norris K. Relationship of C-reactive protein and bone mineral density in community-dwelling elderly females. *J Natl Med Assoc* 2005;97:329-333.
32. Bhupathiraju SN, Alekel DL, Stewart JW, et al. Relationship of circulating total homocysteine and C-reactive protein to trabecular bone in postmenopausal women. *J Clin Densitom* 2007;10:395-403.
33. Koh JM, Khang YH, Jung CH, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int* 2005;16:1263-1271.
34. Kim BJ, Yu YM, Kim EN, Chung YE, Koh JM, Kim GS. Relationship between serum hsCRP concentration and biochemical bone turnover markers in healthy pre- and postmenopausal women. *Clin Endocrinol (Oxf)* 2007;67:152-158.